WHAT IS CLAIMED IS:

1. A method for inserting a cassette into a nucleic acid molecule to produce a nucleic acid-cassette fusion without requiring ligation, said method comprising the steps of:

(a) amplifying two separate regions of a selected nucleic acid molecule, wherein said regions comprise nucleotide sequences flanking a site in the molecule targeted for disruption, whereby the amplification produces a first amplification product of nucleotide sequences upstream of the target site and a second amplification product of nucleotide sequences downstream of the target site;

(b) mixing a cassette to be inserted in the target site with the first amplification product of (a), wherein said cassette comprises a first strand comprising at its 5' end nucleotide sequences which overlap with nucleotides of the first amplification product and at its 3' end nucleotide sequences which overlap with nucleotides of the second amplification product;

(c) amplifying the cassette and the first amplification product of (a), thereby forming a first fusion product consisting of the first amplification product fused to the 5' end of the first strand of the cassette;

(d) mixing the cassette to be inserted in the target site with the second amplification product of (a);

(e) amplifying the cassette and the second amplification product of (a), thereby forming a second fusion product consisting of the second amplification product fused to the 3' end of the first strand of the cassette; and

(f) mixing the first and second fusion products of (c) and (e) and amplifying the fusion products via polymerase chain reaction, thereby producing a nucleic acid cassette fusion comprising the cassette in the target site of the selected nucleic acid molecule.

2. The method according to claim 1, further comprising (g) amplifying

the nucleic acid cassette fusion.

3. The method according to claim 1, wherein the amplification step (a) further comprises performing polymerase chain reaction using a first set of primers, P1 and P2, wherein P1 is a forward primer for the region upstream of the target site and P2 is a reverse primer for the region upstream of the target site and further has a sequence which is complementary to the 5' end of the first strand of the cassette.

4 The method according to claim 3, wherein P1 is about 20bp to about 30 bp and P2 is about 30 bp to about 40 bp.

5. The method according to claim 4, wherein P1 is about 20 bp.

6. The method according to claim 4, wherein P2 is about 40 bp.

7. The method according to claim 2, wherein the amplification step (a) further comprises performing polymerase chain reaction using a second set of primers, P3 and P4, wherein P3 is the forward primer for the region downstream of the target site and has a sequence which is complementary to the 3' end of the cassette, and P4 is the reverse primer for the region downstream of the target site.

8. The method according to claim 7, wherein P3 is about 20 bp to about 30 bp and P4 is about 30 bp to about 50 bp.

9. The method according to claim 8, wherein P3 is about 40 bp in length and P4 is about 20 bp in length.

10. The method according to claim 1, further comprising the step of amplifying the cassette prior to mixture with the amplification products of (c) or (e).

11. The method according to claim 1, wherein the amplification step (c) further comprises performing polymerase chain reaction using a set of primers, P1 and R2, wherein P1 is the forward primer for the region upstream of the target site and R2 is a reverse primer for the cassette.

12. The method according to claim 1, wherein the amplification step (d) further comprises performing polymerase chain reaction using a set of primers, R1 and P4, wherein R1 is the forward primer for the cassette and P4 is the reverse primer for the region downstream of the target site.

13. The method according to claim 1, wherein the amplification of (f) further comprises the steps of:

(i) heating the mixture of (f) for about 5 minutes in the absence of polymerase or primers at about 94EC;

(ii) cooling the heated mixture of (g) to 50EC over about 30 minutes;

(iii) maintaining the mixture at about 50EC for at least about 5 minutes;

(iv) adding a thermostable polymerase to the mixture;

(v) adding a proof-reading polymerase with 3' exonuclease activity to the mixture;

(vi) heating the mixture to about 72EC for about 5 minutes; and

(vii) adding to the mixture primers comprising a 5' forward primer P1 and a 3' reverse primer P4 for the nucleotide sequences downstream of the target site (a) and amplifying using 30 cycles of polymerase chain reaction.

14. The method according to claim 13, wherein said thermostable polymerase is Taq polymerase.

15. The method according to claim 1, wherein said cassette comprises double stranded nucleic acid sequences.

16. The method according to claim 1, wherein said cassette comprises an antibiotic resistance gene.

17. The method according to claim 1, wherein said cassette comprises a DNA sequence selected from the group consisting of promoter, a terminator, and an operator.

18. The method according to claim 1, wherein said cassette comprises a reporter gene.

19. The method according to claim 1, wherein said selected nucleic acid molecule of (a) is a plasmid.

20. The method according to claim 1, wherein said nucleic acid molecule is linear.

21. The method according to claim 1, wherein said nucleic acid sequences are from a gram positive or gram negative bacteria.

22. The method according to claim 21, wherein said nucleic acid sequences are from a Streptococcus or a Staphylococcus bacterium.

23. A nucleic acid-cassette fusion produced according to the method according to claim 1.

24. A method for inserting a cassette into a DNA molecule to produce a

nucleic acid-cassette fusion without requiring ligation, said method comprising the steps of:

(a) providing a selected DNA molecule comprising a first region of DNA sequences upstream of a site targeted for disruption and a second region of DNA sequences downstream of the target site, said first and second region comprising a first strand having a first and second end;

(b) providing a cassette comprising a first strand of DNA, wherein the first strand comprises at its 5' end DNA sequences which overlap with sequences at the second end of the first region, and at its 3' end DNA sequences which overlap with sequences of the first end of the second region;

(c) amplifying the selected DNA sequence using primers for the first and second region, thereby producing amplified first and second regions;

(d) mixing the cassette with the amplified first and second regions;

(e) amplifying the mixture of (d) using polymerase chain reaction, thereby producing without ligation a DNA sequence fusion cassette comprising the first and second regions of the DNA sequence flanking the cassette.

25. The method according to claim 24, wherein the primers for the ends of the first and second regions which overlap with the ends of the cassette contain sequences which are complementary to the sequences of the overlap.

26. The method according to claim 24, wherein the overlap in sequences between the end of the cassette and the second end of the first region is about 10 to about 50 base pairs in length.

27. The method according to claim 24, wherein the overlap in sequences between the end of the cassette and the first end of the second region is about 10 to about 50 base pairs in length.

28. The method according to claim 24, wherein the amplifying step (e) further comprises the steps of:

(i) heating the mixture of (d) for about 5 minutes in the absence of polymerase or primers at about 94EC;

(ii) cooling the heated mixture of (i) to 50EC over about 30 minutes;

(iii) maintaining the mixture at about 50EC for about 5 minutes;

(iv) adding a thermostable polymerase to the mixture;

(v) adding a proof-reading polymerase with 3' exonuclease activity to the mixture;

(vi) heating the mixture to about 72EC for about 5 minutes; and

(vii) adding to the mixture primers comprising a 5' forward primer P1 and a 3' reverse primer P4 for the nucleotide sequence region downstream of the target site.

29. A method for inserting a cassette into a DNA molecule to produce a nucleic acid-cassette fusion without requiring ligation, said method comprising the steps of:

(a) providing a first region of DNA sequences and a second region of DNA sequences, said first and second regions each comprising a first strand having a first and second end;

(b) mixing with the first and second regions a cassette comprising a first strand of DNA, wherein the first strand comprises at its 5' end DNA sequences which overlap with sequences at the second end of the first region, and at its 3' end DNA sequences which overlap with sequences of the first end of the second region;

(c) mixing the cassette with the first and second regions;

(d) heating the mixture of (c) for about 5 minutes in the absence of polymerase or primers at about 94EC;

(e) cooling the heated mixture of (i) to 50EC over about 30 minutes;

(f) maintaining the mixture at about 50EC for about 5 minutes;

(g) adding a thermostable polymerase to the mixture;

(h) adding a proof-reading polymerase with 3' exonuclease activity to the mixture;

(i) heating the mixture to about 72EC for about 5 minutes;

(j) adding to the mixture primers comprising a 5' forward primer P1 for the first region and a 3' reverse primer P4 for the second region, and

(k) amplifying the mixture of (j) using polymerase chain reaction, thereby producing without ligation a DNA sequence fusion cassette comprising the first and second regions of the DNA sequence flanking the cassette;

30. A method of high throughput preparation of disrupted Streptococcus DNA sequences without ligation, said method comprising the step of:

(a) providing a nucleic acid molecule comprising Streptococcus DNA sequences comprising a first region upstream of a site in the Streptococcus DNA targeted for disruption and a second region downstream of the target site, said first and second region each having a first and second end;

(b) providing a cassette comprising at one end, nucleotide sequences which overlap with nucleotides at the second end of the first region, and at its other end, nucleotides which overlap with nucleotides of the first end of the second region;

(c) mixing the nucleic acid molecule with primers for the first and second regions in each of the wells of a plate containing a plurality of reaction wells;

(d) amplifying the first and second regions of the selected Streptococcus DNA sequences;

(e) mixing the cassette and the amplified first and second regions of the Streptococcus DNA sequences; and

(f) amplifying the mixture of (e), thereby producing a nucleic acid fusion molecule comprising the first and second regions of the Streptococcus DNA sequence flanking the cassette.

31. The method according to claim 30, wherein the nucleic acid sequence is a plasmid.

32. The method according to claim 30, further comprising the step of incubating the nucleic acid fusion molecules in the presence of Streptococcus and detecting the presence of the cassette in the target site.